PTO 04-111

International Publication No.
WO 97/06813 A1

for 10/049,783

MUCOSAL PREPARATION CONTAINING PHYSIOLOGICALLY ACTIVE PEPTIDE

Nakayuki Yamamoto and Teruomi Ito

UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. OCTOBER 2003
TRANSLATED BY THE RALPH MCELROY TRANSLATION COMPANY

INTERNATIONAL PATENT OFFICE
WORLD ORGANIZATION FOR INTELLECTUAL PROPERTY
International patent published on
the basis of the Patent Cooperation Treaty
INTERNATIONAL PUBLICATION NO. WO 97/06813 A1

International Patent Classification[6]:	A 61 K 38/00, 9/00
International Filing No.:	PCT/JP96/02277
International Filing Date:	August 12, 1996
International Publication Date:	February 27, 1997
Priority	
Date:	August 15, 1995
Country:	Japan
Number:	Hei 7 [1995]-208010
Designated States:	CA, CN, JP, KR, US, Eurpoean Patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

MUCOSAL PREPARATION CONTAINING PHYSIOLOGICALLY ACTIVE PEPTIDE

[Seirikassei pepuchido o ganyuu suru keinenmaku toyo seizai]

Inventors and Inventors/Applicant (US only):	Nakayuki Yamamoto Teruomi Ito
Applicants (for all Designated States except US):	(Assahi Kasei Kogyo Kabushiki Kaisha) (Hisamitsu Seiyaku Kabushiki Kaisha)

Published with: International Search Report

FOR INFORMATION ONLY

Codes for the identification of PCT contract states on the cover sheets of the documents that publish the international applications in accordance with the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia-Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	South Korea or Republic
KZ	Kazakhstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldavia
MG	Madagascar
MK	Macedonia (former Yugoslavian Republic of Macedonia)
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Vietnam

Industrial application field /1*

This invention pertains to a mucosal preparation characterized in that a physiologically active peptide is blended at least with a sorbefacient capable of promoting absorption of the physiologically active peptide in the nasal mucous membrane and the colon mucous membrane and with a compound having a vasodilatory effect, as well as pertaining to a mucosal preparation of a peptide having medical efficacy via effective mucosal absorption of the physiologically active peptide.

Prior art

Physiologically active peptides such as insulin and calcitonin are generally administered in the form of injection preparations. However, in addition to accompanying pain, visiting a clinic is required for a patient to receive drug administration via injection, and therefore, a preparation that can be administered at home is very desirable.

Also, oral preparations of physiologically active peptides have not reached the stage of practical application because of markedly low absorption via the digestive tract as well as of degradation by protease and the effect of first pass in the liver.

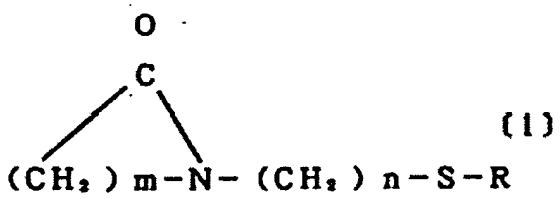
Accordingly, solving such drawbacks is desirable and there have been many attempts to prepare mucosal preparations and increase absorption via the nasal mucosal membrane or the colon mucosal membrane using various sorbefacients. Many mucosal preparations have been reported using sorbefacients that include bile acid salts that have surfactant activity, such as sodium taurocholate, sodium cholate, sodium deoxycholate, sodium chenodeoxycholate, lysine chenodeoxycholate, sodium glycocholate, sodium glycodeoxycholate and lysine taurocholate (Japanese Patent No. Sho 59[1984]-130820, EP Patent No. 115627 Detailed Explanation, Japanese Kokai Patent No. Hei 1[1989]-228919, US Patent No. 5,149,537 Detailed Explanation) and, for examples, surfactants including cationic surfactants such as ethoxylated long-chain /2
amine condensation products and quaternary ammonium compounds such as cetyltrimethylammonium bromide and dodecylmethylammonium [sic; dodecyltrimethylammonium] bromide, anionic surfactants such as alkylbenzenesulfonates, N-acyl-n-alkyltaurates and a-olefin sulfonates, and nonionic surfactants such as polyoxyalkylene higher alcohol ethers and polyalkylene [sic; polyoxyalkylene] alkylphenols (Japanese Kokai Patent No. Hei 4[1992]-247034).

Many mucosal preparations have been reported using sorbefacients via the nasal mucosal membrane and the colon mucosal membrane that include glycyrrhetinates such as diammonium glycyrrhetinate and alkali glycyrrhetinates (mono- or disodium, mono- or dipotassium) (Japanese

* [Numbers in the margin indicate pagination in the foreign document.]

Patent No. Hei 2 [1990]-42027, Japanese Patent No. Hei 3[1995]-5427), cyclodextrins such as a-cyclodextrin, ß-cyclodextrin, ?-cyclodextrin and mono- or di-methylated cyclodextrin (a-, ß- or ?-) (Japanese Patent No. Sho 58 [1983]-189118, EP Patent 94157 Detailed Explanation), O-acyl-L-carnitines having acyl groups of 8-18 carbons (Japanese Patent No. Sho 63-10735, EP Patent 215697 Detailed Explanation), chelating agents, polyacrylic gel substrates and sodium caprylate (US Patent No. 4,476,116 Detailed Explanation).

It is known that azacycloalkane derivatives represented by general formula (1) below:



(1)

(where R represents an alkyl group, m is an integer of 2-4 and n is an integer of 1-15, provided that when n is 1-3, R is an alkyl group of 5-11 carbons) (Japanese Kokai Patent No. Sho 62[1987]-238261) have potent absorption promoting power. /3

There were application examples of bile acid salts and derivatives of fusidic acid such as those reported in J. Japan Diab. Soc., 20(2), 146-152 (1977)/Proc. Nati. Acad. Sci. USA, 82, No. 21:7419-7423 (1985)/Pharm. Res., 9(1), 52-57 (1992). However, it was found that these sorbefacients irritated the nose and caused damage to the mucous membrane, thus their applications were hardly practical.

As such, the preparations using conventional sorbefacients are not flawless when their absorption properties and local irritation are taken into consideration, and none of the preparations have reached the point of practical application. Accordingly, whether the absorption of a peptide drug can be improved or not largely determines the fate of its practical application.

Problem to be solved by the invention

The objective of the present invention lies in providing a mucosal absorption preparation with excellent absorption of physiologically active peptides via the nasal mucous membrane, oral mucous membrane, lung mucous membrane, colon mucous membrane, vaginal mucous membrane, ocular mucous membrane and digestive tract mucous membrane so that sufficient drug efficacy can be expected, as well as having little damaging effect on the membranes.

Means to solve the problems

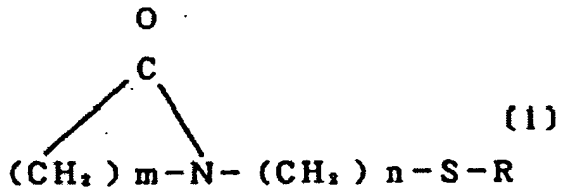
The present inventors repeatedly conducted rigorous investigations aimed at solving the aforementioned problems of developing a mucosal preparation containing physiologically active peptides and having excellent absorption property and safety while having no side effect, and as a result, discovered that absorption of peptides could be significantly increased beyond expectation by incorporating conventional sorbefacients and vasodilatory compounds, such as a calcium channel inhibitor, prostaglandin E1, isosorbide nitrate and nitroglycerin, thus achieving the present invention.

In other words, the objective of the present invention lies in providing a mucosal /4
preparation containing a physiologically active peptide prepared by blending a conventional sorbefacient and vasodilatory compound with the physiologically active peptide. On the other hand, since the compounds having vasodilatory effect have no effect of promoting mucosal absorption in them, or the effect is negligible even if there is some, the result was not predictable from the prior art.

The sorbefacient utilized in the present invention is a general term for all substances that are capable of changing the biological membrane permeation, significantly improving absorption and increasing bioavailability of a drug; sorbefacients have an absorption-promoting effect for a physiologically active peptide in the nasal mucous membrane or colon mucous membrane, and they can be any absorption-promoting substance as long as the absorption of a physiologically active peptide such as insulin via the nasal mucous membrane or the colon mucous membrane can be improved by 200% or more compared with a preparation without the sorbefacient, and preferably it is a sorbefacient that can improve the absorption by 500% or more.

Conventional sorbefacients having absorption-promoting effect for a physiologically active peptide via the nasal mucous membrane or colon mucous membrane can be cited as examples of such sorbefacients. For example, bile acid salts having surfactant activity such as sodium taurocholate, sodium cholate, sodium deoxycholate, sodium chenodeoxycholate, lysine chenodeoxycholate, sodium glycocholate, sodium glycodeoxycholate and lysine taurocholate, and for example, surfactants including cationic surfactants such as ethoxylated long-chain amine condensation products and quaternary ammonium compounds such as cetyltrimethylammonium bromide and dodecylmethylammonium bromide, anionic surfactants such as alkylbenzenesulfonates, N-acyl-n-alkyltaurates and a-olefin sulfonates, and nonionic surfactants such as polyoxyalkylene higher alcohol ethers and polyalkylene alkylphenols, glycyrrhetinates such as diammonium glycyrrhetinate and alkali glycyrrhetinates (mono- or disodium, mono- or dipotassium), cyclodextrins such as a-cyclodextrin, ß-cyclodextrin, ?-cyclodextrin and mono- or /5
di-methylated cyclodextrins (a-, ß- or ?-), O-acyl-L-carnitines having acyl groups of 8-18 carbons, chelating agents, polyacrylic gel substrates and sodium caprylate can be cited.

The preferred sorbefaciant is one or more than one selected from the group comprising bile acid salts, fusidic acid salts, glycyrrhetinates, O-acyl-L-carnitine salts, phospholipids, nonionic surfactants, cyclodextrins, higher fatty acids, 1-alkyl-2-pyrrolidone derivatives, 1-dodecylazacycloheptan-2-one (Azone), bacitracin, sodium azulenesulfonate and azacycloalkane derivatives represented by general formula (1) below:



(1)

(where R represents an alkyl group, m is an integer of 2-4 and n is an integer of 1-15, provided that when n is 1-3, R are alkyl groups of 5-11 carbons).

One or more than one selected from the group comprising sodium taurocholate, sodium glycocholate and sodium deoxycholate can be cited as examples of bile acid salts.

One or more than one selected from the group comprising sodium fusidate and sodium tauro-24,25-dihydrofusidate can be cited as examples of fusidic acid salts.

One or more than one selected from the group comprising glycyrrhetinates and sodium /6
3-2 succinyloxyglycyrrhetinate (Carbenixolone [transliteration]) can be cited as examples of glycyrrhetinates.

O-Acyl-L-carnitine salts having 8-18 carbons in the acyl group can be cited as examples of O-Acyl-L-carnitine salts, and O-ocatnoyl-L-carnitine salts, O-lauroyl-L-carnitine salts and O-palmitoyl-L-carnitine salts can be cited as preferred examples.

One or more than one selected from the group comprising phosphatidyl choline (lecithin), lysophosphatidyl choline (lysolecithin) and lysophosphatidyl glycerol can be cited as examples of phospholipids.

One or more than one selected from the group comprising polyoxyalkylene higher alcohol ethers, polyoxyalkylene alkylphenols and sucrose fatty esters can be cited as examples of nonionic surfactants, and polyoxyethylene(9) lauryl ether (Laureth-9) and polyoxyethylene(24) cholesteryl ether (Choleth-24) can be cited as preferred examples.

One or more than one selected from the group comprising a-cyclodextrin, ß-cyclodextrin, ?-cyclodextrin and di-methyl-ß-cyclodextrin can be cited as examples cyclodextrins.

Higher fatty acids having 16-20 carbons can be cited as examples of higher fatty acids, and one or more than one unsaturated higher fatty acid of 18 carbons selected from the group comprising oleic acid, linoleic acid and linolenic acid can be cited as preferred examples.

One or more than one selected from the group comprising compounds having 4-12 carbons in the alkyl group can be cited as examples of 1-alkyl-2-pyrrolidone derivatives, and compounds having alkyl groups selected from the group comprising butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl and dodecyl groups can be cited as examples in particular. /7

Compounds having straight- or branched-chain alkyl groups including methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl and eicosyl groups as the alkyl group represented by R in general formula (1) can be cited as azacycloalkane derivatives, and 1-[2-(decylthio)ethyl]azacyclopantan-2-one, where R is an alkyl group of 10 carbons, m is 3 and n is 2 (generic name: pyrothiodecane an oily substance), can be cited as the preferred example.

The sorbefacient can be any compound having an absorption-promoting effect in the mucous membrane and is not restricted to the aforementioned specific examples.

The sorbefacients blended in the present invention are preferred to have little irritation to mucous membranes with high safety, and 1-[2-(decylthio)ethyl]azacyclopantan-2-one, sodium glycocholate, lysolecithin, and sodium azulenesulfonate can be cited as examples of such compounds. The particularly preferred sorbefacients for the mucosal preparation of the present invention are 1-[2-(deceylthio)ethyl]azacyclopantan-2-one and lysolecithin. The blending amount of these compounds in the preparation is 0.01-5 wt%.

When an azacycloalkane derivative represented by general formula (1), preferably 1-[2-(decylthio)ethyl]azacyclopantan-2-one, is prepared as an emulsion preparation, glycyrrhetinic acid or a suitable salt thereof, for example its di-potassium salt, is optionally incorporated at 0.01-10% (w/v), or preferably at 0.1-5% (w/v) concentration. /8

The compounds having vasodilatory effect that are utilized in the present invention are compounds having molecular weights of 200-700, and calcium channel inhibitors can be cited as the first examples. In general, calcium channel inhibitors exhibit a vasodilatory effect and prolonging effect on ventricular nodular transport time via inhibiting Ca infiltration into cells, giving efficacy in mitigating hypertension and arrhythmia, and therefore, are widely used in treating various circulatory diseases. Specifically, benzodiazepine derivatives such as diltiazem hydrochloride, phenyalkylamine derivatives such as verapamil hydrochloride and bepridil hydrochloride, dihydropyridine derivatives such as nifedipine hydrochloride, nical dipine hydrochloride and nimodipine hydrochloride, piperazine derivatives such as innarizine and flunarizine and among others, fasudil hydrochloride, can be cited as examples.

Isosorbide nitrate, nitroglycerine and prostaglandin E1 ca be cited as the drugs that have a potent vasodilatory effect. Among them, isosorbide nitrate and nitroglycerin have been widely utilized in injection preparations, oral preparations and tape [sic; transderal] preparations for treating ischemic cardiac disease and angina pectoris. The effect stems from vasodilation via

direct action on vascular smooth muscle, as well as dilation of relatively thick coronary arteries, reducing coronary resistance, and dilation of collateral blood paths, which leads to increasing oxygen supply to ischemic muscle and improving cardiac function. Prostaglandin E1 (PGE1) has a potent vasodilatory effect and inhibitory effect on platelet aggregation and is utilized in clinical application for treating blood-obstructive ulcers accompanying chronic arterial occlusion. Any compound that has this effect can be utilized, and it is not restricted to the aforementioned specific examples.

The compounds having vasodilatory effect that are blended in the preparations of the present invention are various commercially available drugs for treating circulatory diseases, and /9
when incorporated in preparations for local administration to mucous membranes, the amount of incorporation of a vasodilatory compound as an additive is not particularly restricted as long as the amount is capable of effectively aiding the absorption of a physiologically active peptide through the mucous membranes, but the preferred amount is ½ or less the normal minimum amount of the active drug component, and more preferably 1/5 or less. Specifically, the normal minimum amount of a single injection of diltiazem hydrochloride is 10 mg, therefore, the amount of incorporation is preferably ½ or less of that, or more preferably, 1/5 or less of that. The normal minimum amount of a single injection of prostaglandin E1 is 20 μg, therefore, the amount of incorporation is preferably ½ of that (10 μg), or more preferably, 1/5 or less of that (4 μg). The minimum amount in a preparation for one single dose is 0.1 μg or more, preferably 1 μg or more.

The physiologically active peptides utilized in the present invention are physiologically active peptides comprising 3 or more amino acids. Those having molecular weights of 300-10,000 can be cited as preferred examples. Insulin, calcitonin, human PTH (1? 34), calcitonin gene-related peptides (CGRP), angiotensin II, vasopressin, desmopressin acetate, buserelin acetate, goserelin acetate, nafarelin acetate, leuprorelin acetate, somatostatin, glucagon, oxytocin, secretin, leuteinizing hormone-releasing hormone (LH-RH), adrenocorticotropic hormone (ACTH), thyroid hormone-releasing hormone (TRH), thyroid stimulating hormone (TSH), atrial natriuretic peptide (ANP), and derivatives containing synthetic products and semi-synthetic products thereof can be cited as examples of the aforementioned peptides.

Natural calcitonins such as eel calcitonin, salmon calcitonin, human calcitonin, porcine calcitonin and chick calcitonin, and semi-synthetic calcitonins such as ASU^{1-7} eel calcitonin (Elcatonin [transliteration]) and ASU^{1-7} chicken calcitonin can be cited as examples of calcitonins in the present invention. Human insulin, porcine insulin and bovine insulin can be cited as examples of insulin. /10

The particularly preferred peptides in the mucosal preparations of the present invention are Elcatonin and human insulin.

The pharmaceutical preparation of the mucosal preparations of the present invention is explained in the following.

The amount of incorporation of a physiologically active peptide in a preparation of the present invention is optimally selected in accordance with the potency of said peptide and the necessary treatment, and it can be suitably changed in response to the absorption rate of the peptide in nasal mucous membrane. For example, the preferred concentration of the physiologically active peptide in the mucosal preparation as an aqueous solution is 0.000001-5% (w/v), more preferably 0.00001-1% (w/v).

The mucosal preparation of the present invention can be utilized as a drug for application to the nasal mucous membrane, oral mucous membrane, lung mucous membrane, colon mucous membrane, vaginal mucous membrane and ocular mucous membrane, and the preparation can be prepared as a solution, in solid form or semisolid form, but it is more convenient as a spray, aqueous solution for drop administration or as suppository. In the preparation of an aqueous solution or a suppository, water-soluble or oil-soluble substrates such as water, glycerin, propylene glycol, Weetap [transliteration] sol, cocoa butter, soybean oil, medium chain fatty acid triglycerides and polyvinylpyrrolidones can be used.

Such aqueous preparation contains the physiologically active peptide and one or more compounds selected from the aforementioned sorbefacients and one or more compounds selected from the aforementioned vasodilatory agents, and if necessary, pH adjusting agent, isotonic agent, preservative, stabilizer, solubilizer and emulsifier are added, and a given amount of distilled water is then added to dissolve or suspend them to give a preparation of predetermined concentrations. It can also be prepared as an emulsion. Particularly, if there is a stability concern for any of the material in a solution, a solid form can be prepared by further freeze-drying or spray-drying. /11

The pH of the aforementioned preparation is selected from a range so that the pH will not adversely affect the stability of the physiologically active peptide while exerting little damage to the nasal mucous membrane and causing no precipitation. In general the preferred pH is 4-8, and sodium hydroxide, potassium hydroxide, sodium carbonate, sodium bicarbonate, hydrochloric acid, sulfuric acid, or suitable buffer solutions such as phosphate, acetate, lactate and citrate can be added as pH adjusting agents. The osmotic pressure is preferably in an isotonic state, and glycerin, sodium chloride, mannitol or glucose can be added as an isotonic agent if necessary. A preservative may be optionally added, and in general a medically permitted preservative is incorporated. Methyl p-hydroxybenzoate, ethyl p-hydroxybenzoate, propyl p-hydroxybenzoate, butyl p-hydroxybenzoate, benzalkonium chloride, benzethonium chloride, phenylethyl alcohol, chlorobutanol, phenol, cresol, thimerosal, dehydroacetic acid and sorbic acid can be cited as

examples. The suitable concentration of a preservative varies with the type of preservative selected and is in general 0.01-2% (w/v).

In the production method of the preparation of the present invention, the components are dissolved in any order in accordance with respective conventional methods for each component, when preparing an aqueous preparation in the production of the preparations of the present invention. For example, insulin, lysolecithin, prostaglandin E1 and the aforementioned additives pertaining to the present invention are added to a suitable amount of distilled water for injection, which is agitated until all are dissolved, and the pH is adjusted to a desired value using sodium hydroxide or hydrochloric acid as the pH adjusting agent. The obtained aqueous solution is then filtered and sterilized, for example, with a 0.22 μm membrane filter, followed by filling in vials (U-SaVE, product of Sangoban [transliteration] Company), to give an aqueous preparation.

The dose of an aqueous preparation varies with the objective of the drug administration. /12
For example, a nasal mucosal preparation for human administration is sprayed once in one side or once in each side of the nasal cavity, 1-3 doses daily, using a fixed-dose spraying devise (0.05-0.1 mL/press).

Production of suppositories for colon mucosal or vaginal mucosal administration can be carried out using Weetap sol, cocoa butter, Macrogol, propylene glycol or glycerin, if necessary, in accordance with conventional methods.

The method of administration of the preparation of the present invention in general is nebulizing the product onto the mucous membrane via a spraying devise, while aiming at a systemic effect. The preparation of the present invention is administered and adhered to mucous membranes over a wide area so that mucosal penetration is ensured and the peptide is distributed thoughout the whole body. Therefore, the peptide-containing preparation of the present invention can be self-administered by a target patient of the peptide while also not having the drawback of experiencing pain and suffering associated with injection administration. The mucosal preparations can be provided as a nasal mucosal preparation, oral mucosal preparation, lung mucosal preparation, colon mucosal preparation, vaginal mucosal preparation and ocular mucosal preparation.

Detailed explanation of the figures

Figure 1 shows the profile of Elcatonin concentration in blood after nasal administration.

Figure 2 shows the absorption rate (%) with respect to subcutaneous injection (AUC) of Elcatonin .

Figure 3 shows the profile of human insulin concentration in blood after nasal administration.

Figure 4 shows the profile of human insulin concentration in blood after nasal administration.

Figure 5 shows the profile of LH-RH concentration in blood after nasal administration.

Application examples of the invention /13

The present invention is further explained in detail using the below application examples and reference examples, however, they are not to be construed as limiting the present invention. Experimental examples are utilized to show in specifics the effect of the preparations of the present invention.

Reference Example 1

Sodium glycocholate (SGC: Sigma Company, USA) 5 mg was dissolved in 1 mL of pH 6.0 isotonic phosphate buffer solution. A liquid preparation (Control 1) of Elcatonin (400 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and Elcatonin (400 U/vial, lyophilized).

Reference Example 2

Diltiazem hydrochloride (DTZ: Sigma Company, USA) 10 mg was dissolved in 2 mL of pH 6.0 isotonic phosphate buffer solution. A liquid preparation (Control 2) of Elcatonin (400 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and Elcatonin (400 U/vial, lyophilized).

Application Example 1

Diltiazem hydrochloride (DTZ: Sigma Company, USA) 10 mg was dissolved in 2 mL of an aqueous solution of 0.5% sodium glycocholate (pH 6.0 isotonic phosphate buffer solution), to give a solution containing 0.5% SGC and 0.5%DTZ. A liquid preparation of Elcatonin (400 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and Elcatonin (400 U/vial, lyophilized).

Application Example 2

Verapamil hydrochloride (VP: Sigma Company, USA) 10 mg was dissolved in 2 mL of a solution of 0.5% sodium glycocholate obtained by dissolving N-vinyl-2-pyrrolidone (product of Wako Junyaku K.K., Japan) and sodium glycocolate (SGC: Sigma Company, USA) in pH 6.0 /14
isotonic phosphate buffer solution at concentrations of 50 mg and 5 mg, respectively, with respect to 1 mL of the buffer solution, to give a solution containing 0.5% SGC and 0.5% VP. A

liquid preparation of Elcatonin (400 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and Elcatonin (400 U/vial, lyophilized).

Application Example 3

Bepridil hydrochloride (BP: Sigma Company, USA) 10 mg was dissolved in 2 mL of a solution of 0.5% sodium glycocholate prepared by dissolving N-vinyl-2-pyrrolidone (product of Wako Junyaku K.K., Japan) and sodium glycocolate (SGC: Sigma Company, USA) in pH 6.0 isotonic phosphate buffer solution at concentrations of 100 mg and 5 mg, respectively, with respect to 1 mL of the buffer solution, to give a solution containing 0.5% SGC and 0.5% BP. A liquid preparation of Elcatonin (400 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and Elcatonin (400 U/vial, lyophilized).

Application Example 4

Fasudil hydrochloride (FS: Asahi Kasei Kogyo K.K., Japan) 10 mg was dissolved in 2 mL of an aqueous solution of 0.5% sodium glycocholate (pH 6.0 isotonic phosphate buffer solution), to give a solution containing 0.5% SGC and 0.5% FS. A liquid preparation of Elcatonin (400 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and Elcatonin (400 U/vial, lyophilized).

Experimental Example 1

In vivo absorption experiment of Elcatonin in rat (in vivo absorption experiment)

Male Wistar rats (Japan SLC: weight 200-250 g), 4 heads/group, were fasted for one night and put under anesthesia with pentobarbital (nembutal injection solution, product of Dai-Nippon Seiyaku K.K., Japan), followed by cervical incision to insert a polyethylene tube into the trachea. Esophagus incision was then performed to insert a tube into the postnaris. Administration was conducted by applying 25μl of freshly prepared drug preparation using a micropipet. Blood (200 μl) was sampled periodically from the femoral artery before and after drug administration. The blood was centrifuged (15,000 rpm/ 10 min/5°C) and the obtained plasma was stored at -30°C until analysis. /15

For the purpose of comparison, 0.25 mL of 20 U/mL Elcatonin (physiological saline solution) (Comparative Example 1) was administered by subcutaneous injection.

Quantitative method

Determination of blood concentration was conducted in accordance with the RIA method.

Results

Figure 1 shows the Elcatonin concentration in blood over time. Significant increase of Elcatonin concentration in blood was observed in the group (? -?) medicated with the preparation of Application Example 1 blended with 0.5% sodium glycocholate and 0.5% diltiazem hydrochloride, compared with the group (¦ -¦) medicated with Control 1 preparation blended with Elcatonin and 0.5% sodium glycocholate, with the results showing a remarkable increase of Elcatonin via the nasal mucous membrane with a 3.6-fold increase in the area under the curve (AUC) of blood concentration vs. time, compared with that of the group medicated with Control 1 preparation. However, almost no absorption of Elcatonin via the nasal mucous membrane was observed in the group (? -?) medicated with Control 2 preparation containing Elcatonin and diltiazem hydrochloride. In other words, it was found that diltiazem hydrochloride itself possesses no absorption-promoting effect. The results revealed that a blend of sodium glycocholate, which is a sorbefacient, and diltiazem hydrochloride, which is a calcium channel inhibitor, showed extremely excellent absorption-promoting effect. Furthermore, the absorptions were similarly increased as in the case of diltiazem hydrochloride for the groups medicated with the preparations of Application Example 2, Application Example 3 and Application Example 4 blended with verapamil hydrochloride (shown in (O-O) in Figure 1), bepridil hydrochloride (shown in (? -?) in Figure 1) and fasudil hydrochloride (shown in (? -?) in Figure 1), respectively. /16

Figure 2 shows the absorption-promoting effects of various calcium channel inhibitors.

The absorption rates were calculated from the area under the curve (AUC) values of blood concentration vs. time curve of comparative [sic; Control] 1 using the following equation:

$$\text{Absorption rate (\%)} = \frac{\text{AUC(I.N.)}}{\text{AUC(I.N.)}} \times \frac{\text{Dose(S.C.)}}{\text{Dose(S.C.)}} \times 100$$

(Where I.N. denotes intranasal administration and S.C. denotes subcutaneous administration)

Results in Figure 2 showed that the absorption of Elcatonin via the nasal mucous membrane was increased by more than 2-fold with blended calcium channel inhibitor compared with the case where sodium glycocholate was used alone.

Reference Example 3

r-Human insulin (obtained from genetic recombinant yeast: Specific activity 26 U/mg: product of Boehringer Mannheim company, Germany) 10 U/vial (lyophilized) was dissolved in 1

mL of pH 7.4 isotonic phosphate buffer solution to give a liquid preparation (Control 3) of human insulin of 10 U/mL.

Reference Example 4

L-a-Lysolecithin (LPC: Sigma Company, USA) 5 mg was dissolved in 1 mL of pH 7.4 isotonic phosphate buffer solution to prepare a solution containing 0.5% LPC. A liquid preparation (Control 4) of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Application Example 5 /17

Diltiazem hydrochloride (DTZ: Sigma Company, USA) 10 mg was dissolved in 2 mL of an aqueous solution of 0.5% L-a-lysolecithin (pH 7.4 isotonic phosphate buffer solution), to give a solution containing 0.5% LPC and 0.5% DTZ. A liquid preparation of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Application Example 6

Prostandin R20 for injection (prostaglandin E1: PGE1: Ono Yakuhin Kogyo K.K., Japan) 20 μg was dissolved in 2 mL of an aqueous solution of 0.5% L-a-lysolecithin (LPC) (pH 7.4 isotonic phosphate buffer solution), to give a solution containing 0.5% LPC and 0.001% PGE1. A liquid preparation of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Experimental Example 2

In vivo absorption experiment of insulin in rat (in vivo absorption experiment)

Male Wistar rats (Japan SLC: weight 200-250 g), 4 heads/group, were fasted for one night and put under anesthesia with pentobarbital (nembutal injection solution, product of Dai-Nippon Seiyaku K.K., Japan), followed by cervical incision to insert a polyethylene tube into the trachea to insure the integrity of the trachea. Esophagus incision was then performed to insert a tube into the postnaris, while the tip of the tube was sealed with cotton to prevent the drug solution from leaking from the nasal cavity into the esophagus. Administration was conducted by applying 25μl of a freshly prepared drug preparation to the right nasal cavity using a micropipet. Blood (200μl) was sampled periodically from the femoral artery before and after drug administration. The blood was centrifuged (15,000 rpm/ 10 min/5°C) and the obtained plasma was stored at -30°C until analysis.

Quantitative analysis /18

Determination of blood concentration was conducted in accordance with the EIA method based on one-step sandwich method using two types of monoclonal antibodies with human insulin assaying reagent (Boehringer Mannheim Company, Germany).

Results

Figure 3 shows the insulin concentration in blood over time. The results in the figure show that almost no absorbed insulin via the nasal mucous membrane was observed in the group (? -?) medicated with the Control 3 preparation, which is an aqueous solution of insulin. A significant increase in absorption was observed when insulin, 0.5% diltiazem hydrochloride and 0.001% PGE1 were added, compared with the group (O-O) medicated with the Control 4 preparation containing insulin and 0.5% L-a-lysolecithin (LPC). The results show a statistically significant increase of insulin absorption via the nasal mucous membrane in the group medicated with the preparation of Application Example 5 (¦ -¦) with added 0.5% diltiazem hydrochloride and in the group medicated with the preparation of Application Example 6 (? -?) with added 0.001% PGE1, with the area under the curve (AUC) of the blood concentration vs. time increasing 1.7-fold and 1.8-fold, respectively, compared with the group medicated with the preparation of Control 4 preparation.

Application Example 7

1.	Elcatonin	1000 U
2.	Sodium glycocholate	5 mg
3.	Diltiazem hydrochloride	5 mg
4.	Glycerin	22 mg
5.	Methyl p-hydroxybenzoate	1 mg
6.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 5.5
7.	Distilled water for injection	Balance for total quantity of 1 mL

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration.

3 mL each [sic] of the obtained solution were sterilized and filtered (0.22 μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 1000 U/mL of Elcatonin, and one press on the adaptor precisely discharged a nebulized dose of 100 U. /19

Application Example 8

1.	Elcatonin	1000 U
2.	L-a-Lysolecithin	5 mg
3.	Prostaglandin E1	10 μg
4.	Glycerin	22 mg
5.	Methyl p-hydroxybenzoate	1 mg
6.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 5.5
7.	Distilled water for injection	Balance for total quantity of 1 mL

/20

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22 μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 1000 U/mL of Elcatonin and one press on the adaptor precisely discharged a nebulized dose of 100 U

Application Example 9

1.	Elcatonin	1000 U
2.	L-a-Lysolecithin	5 mg
3.	Isosorbide nitrate	0.1 mg
4.	Glycerin	17.6 mg
5.	D-Sorbitol	10 mg
6.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 5.5
7.	Distilled water for injection	Balance for total quantity of 1 mL

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 1000 U/mL of Elcatonin and one press on the adaptor precisely discharged a nebulized dose of 100 U.

Application Example 10

1.	r-Human insulin	100 U
2.	Sodium glycocholate	5 mg
3.	Diltiazem hydrochloride	5 mg
4.	Glycerin	22 mg

/21

5.	Methyl p-hydroxybenzoate	1 mg
6.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 6.0
7.	Distilled water for injection	Balance for total quantity of 1 mL

The mucosal preparation of the above composition(1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22 μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 100 U/mL of r-human insulin and one press on the adaptor precisely discharged a nebulized dose of 10 U.

Application Example 11

1.	r-Human insulin	100 U
2.	L-a-Lysolecithin	5 mg
3.	Prostaglandin E1	10μg
4.	Glycerin	22 mg
5.	Benzalkonium chloride	0.1 mg
6.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 6.0
7.	Distilled water for injection	Balance for total quantity of 1 mL

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 100 U/mL of r-human insulin and one press on the adaptor precisely discharged a nebulized dose of 10 U.

Application Example 12

1.	r-Humanl insulin	100 U
2.	L-a-Lysolecithin	5 mg
3.	Isosorbide nitrate	0.1 mg
4.	Glycerin	17.6 mg
5.	Benzalkonium chloride	0.1 mg
6.	D-Sorbitol	10 mg
7	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 6.0
8.	Distilled water for injection	Balance for total quantity of 1 mL

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22 μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 100 U/mL of r-human insulin and one press on the adaptor precisely discharged a nebulized dose of 10 U.

Reference Example 5 /22

Sorbefacient pyrothiodecane (1-[2-(decylthio)ethyl]azacyclopantan-2-one (product of Hisamitsu Seiyaku K.K., Japan)), emulsifier dipotassium glycyrrhetinate and isotonic agent glycerin were dissolved in a suitable amount of distilled water at 5 mg, 10 mg and 22 mg, respectively, with respect to a final solution of 1 mL after volume adjustment, which was subjected to ultrasound treatment to give a homogeneous solution. Next, the pH was adjusted with 1N sodium hydroxide to 6.0 and the volume was adjusted so that the solution contained 0.5% pyrothiodecane. A liquid preparation (Control 5) of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Reference Example 6

With respect to 1 mL of solution after volume adjustment, prostaglandin E1 (PGE1: prostantin R20 for injection, Ono Yakuhin Kogyo K.K., Japan) 0.05 mg was dissolved, followed by adding 25.7 mg of isotonic agent glycerin, and the pH was adjusted to 6.0 using 1N sodium hydroxide, followed by volume adjustment, to give a solution containing 0.005% PGE1. A liquid preparation (Control 6) of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Application Example 13

Sorbefacient pyrothiodecane, emulsifier dipotassium glycyrrhetinate, isotonic agent glycerin and vasodilator prostaglandin E1 (PGE1: prostantin R20 for injection, Ono Yakuhin Kogyo K.K., Japan) were dissolved in a suitable amount of distilled water at 5 mg, 10 mg 22 mg and 0.01 mg, respectively, with respect to a final solution of 1 mL after volume adjustment, which was subjected to ultrasound treatment to prepare a homogeneous solution. Next, the pH was adjusted with 1N sodium hydroxide to 6.0 and the volume was adjusted so that the solution contained 0.5% pyrothiodecane and 0.001% PGE1. A liquid preparation of human insulin /23

(10 U/mL) was prepared by mixing and dissolving together1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Application Example 14

Sorbefacient pyrothiodecane, emulsifier dipotassium glycyrrhetinate, isotonic agent glycerin and vasodilator isosorbide nitrate (ISDN: Nitrol for injection, product of Eizai K.K., Japan) were dissolved in a suitable amount of distilled water at 5 mg, 10 mg 17.6 mg and 0.1 mg, respectively, with respect to a final solution of 1 mL after volume adjustment, which was subjected to ultrasound treatment to prepare a homogeneous solution. Next, the pH was adjusted with 1N sodium hydroxide to 6.0 and the volume was adjusted so that the solution contained 0.5% pyrothiodecane and 0.01% ISDN. A liquid preparation of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Application Example 15 /24

Sorbefacient pyrothiodecane, emulsifier dipotassium glycyrrhetinate, isotonic agent glycerin and vasodilator isosorbide nitrate (ISDN: Nitrol for injection, product of Eizai K.K., Japan) were dissolved in a suitable amount of distilled water at 5 mg, 10 mg 13.2 mg and 0.2 mg, respectively, with respect to a final solution of 1 mL after volume adjustment, which was subjected to ultrasound treatment to prepare a homogeneous solution. Next, the pH was adjusted with 1N sodium hydroxide to 6.0 and the volume was adjusted so that the solution contained 0.5% pyrothiodecane and 0.02% ISDN. A liquid preparation of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized) together.

Application Example 16

Sorbefacient pyrothiodecane, emulsifier dipotassium glycyrrhetinate, isotonic agent glycerin and vasodilator nitroglycerin (Milisurol [transliteration] for injection, product of Nihon Kayaku K.K., Japan) were dissolved in a suitable amount of distilled water at 5 mg, 10 mg, 17.6 mg and 0.1 mg, respectively, with respect to a final solution of 1 mL after volume adjustment, which was subjected to ultrasound treatment to prepare a homogeneous solution. Next, the pH was adjusted with 1N sodium hydroxide to 6.0 and the volume was adjusted so that the solution contained 0.5% pyrothiodecane and 0.01% nitroglycerin. A liquid preparation of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Experimental Example 3

In vivo absorption experiment of insulin in rat (2) (in vivo absorption experiment)

Male Wistar rats (Japan SLC: weight 200-250 g), 4 heads/group, were fasted for one night and put under anesthesia with pentobarbital (nembutal injection solution, product of Dai-Nippon Seiyaku K.K., Japan), followed by cervical incision to insert a polyethylene tube into the trachea to insure the integrity of the trachea. Esophagus incision was then performed to insert a tube into the postnaris, while the tip of the tube was sealed with cotton to prevent the drug solution from leaking from the nasal cavity into the esophagus. Administration was conducted by applying 25μl of freshly prepared drug preparation to the right nasal cavity using a micropipet. Blood (200μl) was sampled periodically from the femoral artery before and after drug administration. The blood was centrifuged (15,000 rpm/ 10min/5°C) and the obtained plasma was stored at -30°C until analysis.

Quantitative analysis

Determination of blood concentration was conducted in accordance with the EIA method based on one-step sandwich method using two types of monoclonal antibodies with human insulin assaying reagent (Boehringer Manheim Company, Germany). /25

Results

Figure 4 shows the insulin concentrations in blood over time. The results in the figure showed that almost no absorption via the nasal mucous membrane was observed in the group (? -?) medicated with Control 3 preparation, which is an aqueous solution of insulin. Similarly, almost no absorption via the nasal mucous membrane was observed in the group (O-O) medicated with Control 6 preparation containing insulin and 0.005% PGE1. Compared with the group medicated with Control 5 preparation (Δ-Δ) of the prior art containing insulin and 0.5% pyrothiodecane, significant increases of insulin absorption via the nasal mucous membrane were observed in the group medicated with the preparation of Application Example 13 of the present invention (¦ -¦) containing insulin, 0.5% pyrothiodecane and 0.001% PGE1, in the group medicated with the preparation of Application Example 14 of the present invention (? -?) with added 0.01% ISDN, in the group medicated with the preparation of Application Example 15 of the present invention (? -?) with added 0.02% ISDN and in the group medicated with the preparation of Application Example 16 of the present invention (+-+) with added 0.01% nitroglycerin. The areas under the curve (AUC; 0-2 h) of blood concentration vs. time of these groups increased significantly by about 1.7-fold, 1.9-fold, 2.0-fold and 2.4-fold, respectively, compared with the group medicated with Control 6 preparation.

Application Example 17

1.	r-Human insulin	100 U
2.	Pyrothiodecane	5 mg
3.	PGE1	0.005 mg
4.	Dipotassium glycyrrhetinate	10 mg
5	Glycerin	22 mg
6.	Benzalkonium chloride	0.1 mg
7.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 6.0
8.	Distilled water for injection	Balance for total quantity of 1 mL

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration. /26

3 mL each of the obtained solution were sterilized and filtered (0.22μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 100 U/mL of r-human insulin and one press on the adaptor precisely discharged a nebulized dose of 10 U.

Application Example 18

1.	r-Human insulin	500 U
2.	Pyrothiodecane	5 mg
3.	PGE1	0.01 mg
4.	Dipotassium glycyrrhetinate	10 mg
5	Glycerin	22 mg
6.	Benzalkonium chloride	0.1 mg
7.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 6.0
8.	Distilled water for injection	Balance for total quantity of 1 mL

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22 μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 100 U/mL of r-human insulin and one press on the adaptor precisely discharged a nebulized dose of 10 U.

Application Example 19

1.	r-Human insulin	100 U

2.	Pyrothiodecane	5 mg
3.	Isosorbide nitrate	0.1 mg
4.	Dipotassium glycyrrhetinate	10 mg
5	Glycerin	17.6 mg
6.	Benzalkonium chloride	0.1 mg
7.	D-Sorbitol	10 mg
8.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 6.0
9.	Distilled water for injection	Balance for total quantity of 1 mL

/27

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 100 U/mL of r-human insulin and one press on the adaptor precisely discharged a nebulized dose of 10 U.

Application Example 20

1.	r-Human insulin	100 U
2.	Pyrothiodecane	5 mg
3.	Isosorbide nitrate	0.2 mg
4.	Dipotassium glycyrrhetinate	10 mg
5	Glycerin	13.2 mg
6.	Benzalkonium chloride	0.1 mg
7.	D-Sorbitol	20 mg
8.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 6.0
9.	Distilled water for injection	Balance for total quantity of 1 mL

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22 μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 100 U/mL of r-human insulin and one press on the adaptor precisely discharged a nebulized dose of 10 U.

/28

Application Example 21

1.	r-Human insulin	100 U

2.	Pyrothiodecane	5 mg
3.	Nitroglycerin	0.1 mg
4.	Dipotassium glycyrrhetinate	10 mg
5	Glycerin	17.6 mg
6.	Benzalkonium chloride	0.1 mg
7.	D-Mannitol	10 mg
8.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 6.0
9.	Distilled water for injection	Balance for total quantity of 1 mL

The mucosal preparation of the above composition(1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 100 U/mL of r-human insulin and one press on the adaptor precisely discharged a nebulized dose of 10 U.

Reference Example 7

With respect to 1 mL of solution after volume adjustment, isosorbide nitrate (ISDN: Nitrol for injection, product of Eizai K.K., Japan) 0.1 mg was dissolved, followed by adding 25.7 mg of isotonic agent glycerin, and the final pH was adjusted to 6.0 using 1N sodium hydroxide, followed by volume adjustment, to give a solution containing 0.01% ISDN. A liquid preparation of LH-RH (100 μg/mL) (Control 7) was prepared by mixing and dissolving together 1 mL of said solution and 100 μg/vial luteinizing hormone-releasing hormone (LH-RH: Sigma Company, USA, lyophilized).

Reference Example 8 /29

Sorbefacient pyrothiodecane, emulsifier dipotassium glycyrrhetinate and isotonic agent glycerin were dissolved in a suitable amount of distilled water at 5 mg, 10 mg and 22 mg, respectively, with respect to a final solution of 1 mL after volume adjustment, which was subjected to ultrasound treatment to prepare a homogeneous solution. Next, the pH was adjusted with 1N sodium hydroxide to 6.0 and the volume was adjusted so that the solution contained 0.5% pyrothiodecane. A liquid preparation of LH-RH (100 μg/mL) (Control 8) was prepared by mixing and dissolving together 1 mL of said solution and 100 μg/vial luteinizing hormone-releasing hormone (LH-RH: Sigma Company, USA, lyophilized).

Application Example 22

Sorbefacient pyrothiodecane, emulsifier dipotassium glycyrrhetinate, isotonic agent glycerin and vasodilator isosorbide nitrate (ISDN: Nitrol for injection, product of Eizai K.K., Japan) were dissolved in a suitable amount of distilled water at 5 mg, 10 mg 17.6 mg and 0.1 mg, respectively, with respect to a final solution of 1 mL after volume adjustment, which was subjected to ultrasound treatment to prepare a homogeneous solution. Next, the pH was adjusted with 1N sodium hydroxide to 6.0 and the volume was adjusted so that the solution contained 0.5% pyrothiodecane and 0.01% ISDN. A liquid preparation of human insulin [sic; LH-RH] (100 μg/mL) was prepared by mixing and dissolving together 1 mL of said solution and 100 μg/vial luteinizing hormone-releasing hormone (LH-RH: Sigma Company, USA, lyophilized).

Experimental Example 4

In vivo absorption experiment of LH-RH in rat (in vivo absorption experiment)

Male Wistar rats (Japan SLC: weight 200-250 g), 4 heads/group, were fasted for one night and put under anesthesia with pentobarbital (nembutal injection solution, product of Dai-Nippon Seiyaku K.K., Japan), followed by cervical incision to insert a polyethylene tube /30
into the trachea to insure the integrity of the trachea. Esophagus incision was then performed to insert a tube into the postnaris, while the tip of the tube was sealed with cotton to prevent the drug solution from leaking from the nasal cavity into the esophagus. Administration was conducted by applying 10 μg/0.1 mg/kg of freshly prepared drug preparation to the right nasal cavity using a micropipet. Blood (200μl) was sampled periodically from the femoral artery before and after drug administration. The blood was centrifuged (15,000 rpm/ 10min/5°C) and the obtained plasma was stored at -30°C until analysis.

Quantitative analysis

Determination of blood concentration was conducted in accordance with the EIA method using the LH-RH assaying reagent (Peninsula Company, USA).

Results

Figure 5 shows the LH-RH concentrations in blood over time. The results in the figure showed that almost no absorption via the nasal mucous membrane was observed in the group (?-?) medicated with Control 7 preparation containing LH-RH and 0.01% ISDN. An increase of LH-RH absorption via the nasal mucous membrane was observed in the group medicated with the preparation of Application Example 22 of the present invention (¦ -¦) containing insulin [sic: LH-RH], 0.5% pyrothiodecane and 0.01% ISDN, compared with the group medicated with Control 8 preparation (? -?) of the prior art containing LH-RH and 0.5% pyrothiodecane. The

area under the curve (AUC; 0-2 h) of blood concentration vs. time of the group was increased by about 1.5-fold, compared with the group medicated with Control 8 preparation.

Application Example 23

1.	LH-RH	5 mg
2.	Pyrothiodecane	5 mg
3.	Isosorbide nitrate	0.1 mg
4.	Dipotassium glycyrrhetinate	10 mg
5	Glycerin	17.6 mg
6.	Benzalkonium chloride	0.1 mg
7.	D-Sorbitol	10 mg
8.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 6.0
9.	Distilled water for injection	Balance for total quantity of 1 mL

/31

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 5 mg/mL of LH-RH and one press on the adaptor precisely discharged a nebulized dose of 0.5 mg.

Reference Example 9

Isosorbide nitrate (ISDN: Nitrol for injection, product of Eizai K.K, Japan) 0.2 mg was dissolved in 1 mL of pH 7.4 isotonic phosphate buffer solution, to give a solution containing 0.02% ISDN. A liquid preparation (Control 9) of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Application Example 24

Isosorbide nitrate (ISDN: Nitrol for injection, product of Eizai K.K, Japan) 0.1 mg was dissolved in 1 mL of an aqueous solution of 0.5% L-a-lysolecithin (pH 7.4 isotonic phosphate buffer solution), to give a solution containing 0.5% LPC and 0.01% ISDN. A liquid preparation of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Application Example 25 /32

Nitroglycerin (Milisurol for injection, product of Nihon Kayaku K.K, Japan) 0.1 mg was dissolved in 1 mL of an aqueous solution of 0.5% L-a-lysolecithin (pH 7.4 isotonic phosphate buffer solution), to give a solution containing 0.5% LPC and 0.01% nitroglycerin. A liquid preparation of human insulin (10 U/mL) was prepared by mixing and dissolving together 1 mL of said solution and r-human insulin (10 U/vial, lyophilized).

Experimental Example 5

In vivo absorption experiment of insulin in rat (3) (in vivo absorption experiment)

Male Wistar rats (Japan SLC: weight 200-250 g), 4 heads/group, were fasted for one night and put under anesthesia with pentobarbital (nembutal injection solution, product of Dai-Nippon Seiyaku K.K., Japan), followed by cervical incision to insert a polyethylene tube into the trachea to insure the integrity of the trachea. Esophagus incision was then performed to insert a tube into the postnaris, while the tip of the tube was sealed with cotton to prevent the drug solution from leaking from the nasal cavity into the esophagus. Administration was conducted by applying 25μl of freshly prepared drug preparation to the right nasal cavity using a micropipet. Blood (200μl) was sampled periodically from the femoral artery before and after drug administration. The blood was centrifuged (15,000 rpm/ 10 min/5°C) and the obtained plasma was stored at -30°C until analysis.

Quantitative analysis

Determination of blood concentration was conducted in accordance with the EIA method based on one-step sandwich method using two types of monoclonal antibodies with human insulin assaying reagent (Boehringer Manheim Company, Germany).

Results

The results showed that almost no absorption via the nasal mucous membrane was observed in the group medicated with Control 3 preparation, which was an aqueous solution of insulin. Similarly, almost no absorption via the nasal mucous membrane was observed in the group medicated with Control 9 preparation containing insulin and 0.02% isosorbide nitrate (ISDN). compared with the group medicated with Control 4 preparation of prior art /33 containing insulin and 0.5% L-a-lysolecithin (LPC), significant increases of insulin absorption were observed in the group medicated with the preparation of Application Example 24 of the present invention containing insulin and 0.5% LPC added with 0.01% ISDN or 0.01% nitroglycerin and in the group medicated with the preparation of Application Example 25 of the present invention added with 0.01% nitroglycerin. The areas under the curve (AUC; 0-2h) of

blood concentration vs. time of these groups increased significantly by about 1.7-fold and 1.5-fold, respectively, compared with the group medicated with Control 4 preparation.

Application Example 26

1.	r-Human insulin	100 U
2.	L-a-Lysolecithin	5 mg
3.	Nitroglycerin	0.1 mg
4.	Glycerin	17.6 mg
5.	Benzalkonium chloride	0.1 mg
6.	D-Mannitol	10 mg
7.	Hydrochloric acid/sodium hydroxide	Appropriate amount to adjust the pH to 6.0
8.	Distilled water for injection	Balance for total quantity of 1 mL

The mucosal preparation of the above composition (1 mL) was prepared at the above concentration.

3 mL each of the obtained solution were sterilized and filtered (0.22 μm membrane filter), followed by filling into a vial for mechanical spray purpose under sterile conditions, to give a finished product. Said product contained 100 U/mL of r-human insulin and one press on the adaptor precisely discharged a nebulized dose of 10 U.

Effect of the invention /34

The mucosal preparation of the present invention containing a physiologically active peptide gives the effect of increasing absorption via the nasal mucous membrane, with a minimum amount of incorporation of a sorbefacient, while exerting no damaging effect to the mucous membrane.

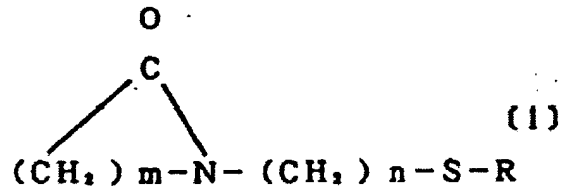
Claims /35

1. A mucosal preparation characterized in that a physiologically active peptide is blended at least with a sorbefacient capable of promoting absorption of the physiologically active peptide in the nasal mucous membrane and colon mucous membrane and with a compound having vasodilatory effect.

2. The mucosal preparation according to the description in Claim 1, [characterized] in that the sorbefacient having a promoting effect on absorption of the physiologically active peptide with respect to the nasal mucous membrane and colon mucous membrane shows an absorption-promoting effect that is capable of improving the absorption rate via the nasal

mucous membrane or colon mucous membrane by more than 200% compared with a preparation without a sorbefacient when insulin is utilized as the physiologically active peptide.

3. The mucosal preparation according to the description in Claim 1, [characterized] in that the sorbefaciant is one or more than one selected from the group comprising bile acid salts, fusidic acid salts, glycyrrhetinates, O-acyl-L-carnitine salts, phospholipids, nonionic surfactants, cyclodextrins, higher fatty acids, 1-alkyl-2-pyrrolidone derivatives, 1-dodecylazacycloheptan-2-one, bacitracin, sodium azulenesulfonate and azacycloalkane derivatives represented by general formula (1) below:



(1)

(where R represents a alkyl group, m is a integer of 2-4 and n is an integer of 1-15, provided that when n is 1-3, R is an alkyl group of 5-11 carbons).

4. The mucosal preparation according to the description in Claim 3, [characterized] in /36 that the bile acid salt is one or more than one selected from the group comprising sodium taurocholate, sodium glycocholate and sodium deoxycholate.

5. The mucosal preparation according to the description in Claim 3, [characterized] in that the fusidic acid salt is one or more than one selected from the group comprising sodium fusidate and sodium tauro-24,25-dihydrofusidate.

6. The mucosal preparation according to the description in Claim 3, [characterized] in that the glycyrrhetinate is one or more than one selected from the group comprising sodium glycyrrhetinate and 3-succinyloxyglycyrrhetinate (Carbenoxolone).

7. The mucosal preparation according to the description in Claim 3, [characterized] in that the O-Acyl-L-carnitine salt is an O-Acyl-L-carnitine salt having 8-18 carbons.

8. The mucosal preparation according to the description in Claim 3, [characterized] in that the O-Acyl-L-carnitine salt is one or more than one kind selected from O-ocatnoyl-L-carnitine salts, O-lauroyl-L-carnitine salts and O-palmitoyl-L-carnitine salts.

9. The mucosal preparation according to the description in Claim 3, [characterized] in that the phospholipid is one or more than one selected from the group comprising phosphatidyl choline (lecithin), lysophosphatidyl choline (lysolecithin) and lysophosphatidyl glycerol.

10. The mucosal preparation according to the description in Claim 3, [characterized] in /37

that the nonionic surfactant is one or more than one selected from the group comprising polyoxyalkylene higher alcohol ethers, polyoxyalkylene alkyl phenols and sucrose fatty esters.

11. The mucosal preparation according to the description in Claim 3, [characterized] in that the nonionic surfactant is one or more than one selected from the group comprising polyoxyethylene(9)lauryl ether (Laureth-9) and polyoxyethylene(24)cholesteryl ether (Choleth-24).

12. The mucosal preparation according to the description in Claim 3, [characterized] in that the cyclodextrin is one or more than one selected from the group comprising a-cyclodextrin, ß-cyclodextrin, ?-cyclodextrin and di-methyl-ß-cyclodextrin.

13. The mucosal preparation according to the description in Claim 3, [characterized] in that the higher fatty acids are fatty acids having 16-20 carbons.

14. The mucosal preparation according to the description in Claim 13, [characterized] in that the higher fatty acid having 16-20 carbons is one or more than one kind of an unsaturated higher fatty acids of 18 carbons selected from the group comprising oleic acid, linoleic acid and linolenic acid.

15. The mucosal preparation according to the description in Claim 3, [characterized] in that the 1-alkyl-2-pyrrolidone derivative is one or more than one selected from the group comprising compounds having 4-12 carbons in the alkyl group.

16. The mucosal preparation according to the description in Claim 15, [characterized] in that the alkyl group is one or more than one selected from the group comprising butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl and dodecyl groups.

17. The mucosal preparation according to the description in Claim 3, [characterized] in /38
that azacycloalkane derivative represented by general formula (1) is 1-[2-(decylthio)ethyl]azacyclopantan-2-one, where R is an alkyl group of 10 carbons, m is 3 and n is 2.

18. The mucosal preparation according to the description in Claim 1, [characterized] in that the amount of incorporation of the sorbefacient is 0.01-5 wt% in the mucosal preparation.

19. The mucosal preparation according to the description in Claim 1, [characterized] in that the compound having vasodilatory effect is one or more than one selected from the group comprising calcium channel inhibitors having molecular weights of 200-700, prostaglandin E1, isosorbide nitrate and nitroglycerin.

20. The mucosal preparation according to the description in Claim 19, [characterized] in that the calcium channel inhibitor is diltiazem hydrochloride, berapamil hydrochloride, vepridil hydrochloride, nifedipine hydrochloride, nicaldipine hydrochloride and fasudil hydrochloride.

21. The mucosal preparation according to the description in Claim 1, [characterized] in that the amount of incorporation of the compound having vasodilatory effect is under ½ the normal minimum amount of the active drug component.

22. The mucosal preparation according to the description in Claim 1, [characterized] in that the molecular weight of the physiologically active peptide is 300-10,000.

23. The mucosal preparation according to the description in Claim 1, [characterized] in that the physiologically active peptides are peptides selected from the group comprising insulin, calcitonin, human PTH (1? 34), calcitonin gene related peptides (CGRP), angiotensin II, vasopressin, desmopressin acetate, buserelin acetate, goserelin acetate, nafarelin acetate, leuprorelin acetate, somatostatin, glucagon, oxytocin, secretin, leuteinizing hormone releasing hormone (LH-RH), adrenocorticotropic hormone (ACTH), thyroid hormone-releasing hormone (TRH), thyroid stimulating hormone (TSH), atrial natriuretic peptide (ANP), and derivatives containing synthetic products and semisynthetic products thereof. /39

24. The mucosal preparation according to the description in Claim 23, [characterized] in that the calcitonin is selected from the group comprising eel calcitonin, salmon calcitonin, porcine calcitonin, human calcitonin, and chick calcitonin.

25. The mucosal preparation according to the description in Claim 24, [characterized] in that the eel calcitonin is ASU^{1-7} eel calcitonin (Elcatonin).

26. The mucosal preparation according to the description in Claim 23, in that the insulin is selected from the group comprising human insulin, porcine insulin and bovine insulin.

27. The mucosal preparation according to the description in Claim 1, in that the mucosal preparation is at least one selected from nasal mucosal preparation, oral mucosal preparation, lung mucosal preparation, colon mucosal preparation, vaginal mucosal preparation and ocular mucosal preparation.

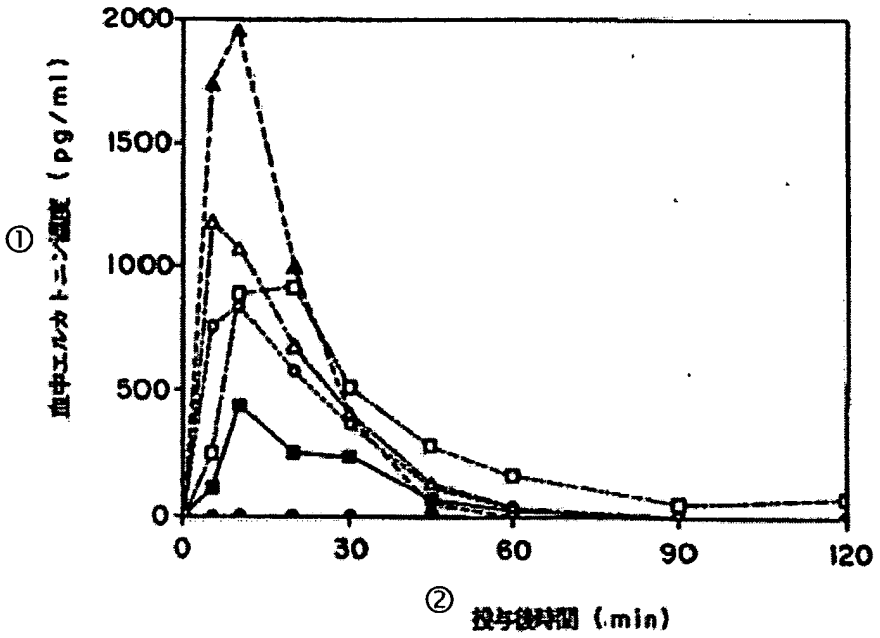


Figure 1

Key: 1 Elcatonin concentration in blood
2 Elapsed time after administration

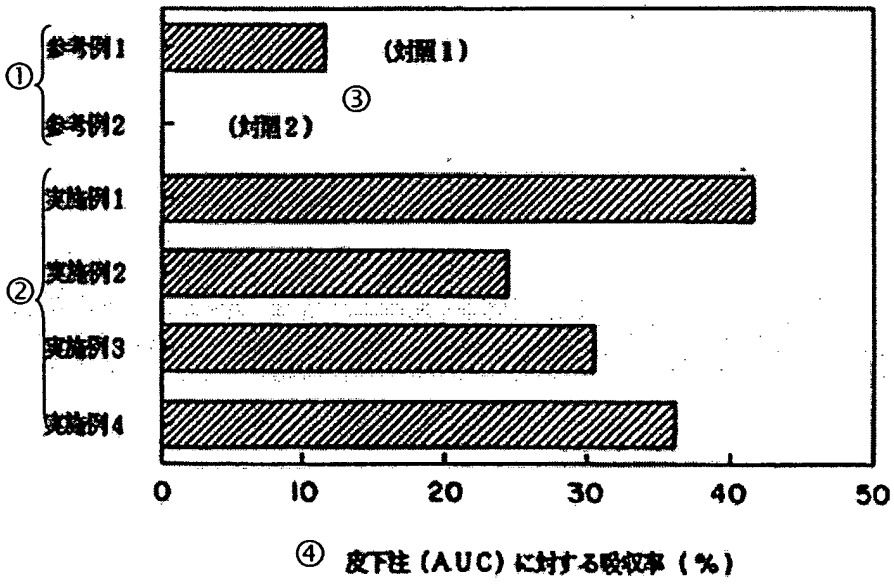


Figure 2

Key: 1 Reference example
2 Application example

3 Control ___
4 Absorption rate with respect to subcutaneous injection (AUC)

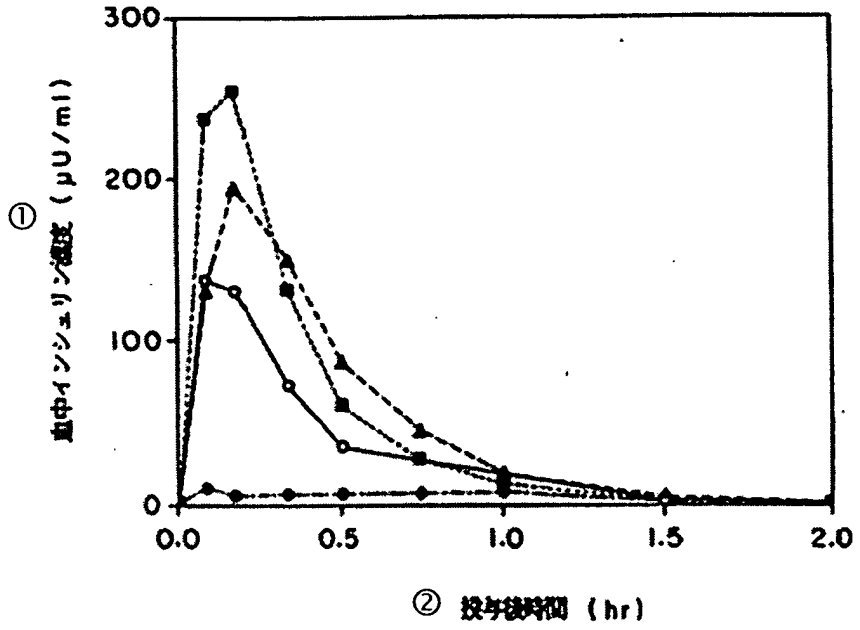


Figure 3

Key: 1 Insulin concentration in blood
2 Elapsed time after administration

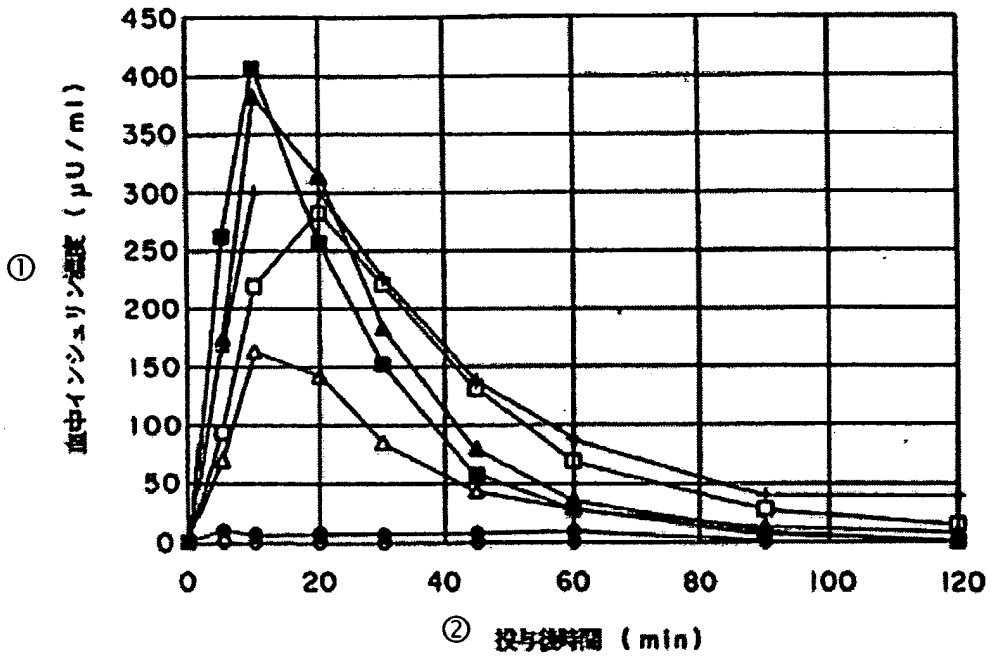


Figure 4

Key: 1 Insulin concentration in blood
2 Elapsed time after administration

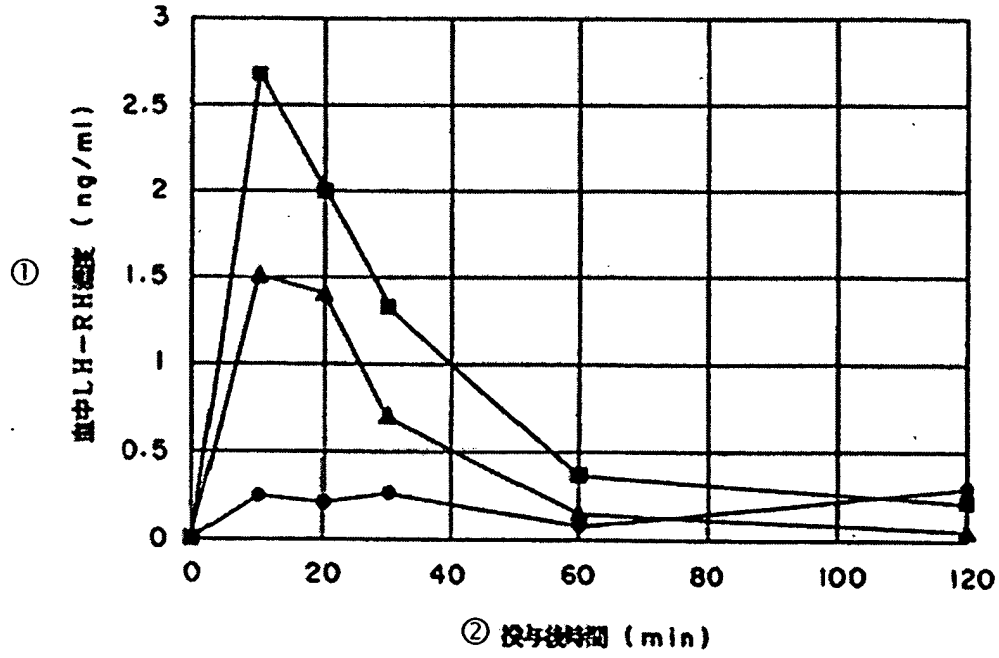


Figure 5

Key: 1 LH-RH concentration in blood
2 Elapsed time after administration

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP96/02277

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl[6] A61K38/00, A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int. Cl[6] A61K38/00, A61K9/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Database WPIL on DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP, 58-189118, A (Takeda Chemical Ind. K.K.), November 4, 1983 (04. 11. 83) & US, 4659696, A & EP, 94157, A	1-3, 12, 18, 22-27
A	JP, 3-505462, A (Ney U. M.), November 28, 1991 (28. 11. 91) & EP, 433402, A & WO, 90/11769	1, 19-23
A	EP, 566135, A (Takeda Chemical Ind. K.K.), October 20, 1993 (20. 10. 93) & JP, 6-9424, A & US, 5482706, A	1, 2, 19 - 27
A	JP, 1-501550, A (Novo-Nordisk AS.), June 1, 1989 (01. 06. 89) & EP, 272097, A & WO, 88-4556 & US, 4179079, A	1-3, 9, 18, 22, 23, 27
A	JP, 59-130820, A (Armour Pharm Co.), July 27, 1984 (27. 07. 84) & EP, 115627, A	1, 3, 4, 10, 18, 22 - 27
A	JP, 4-230223, A (Sandoz AG.),	1, 3, 4,

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
October 30, 1996 (30. 10. 96)	November 12, 1996 (12. 11. 96)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP96/02277

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	August 19, 1992 (19. 08. 92) & EP, 462071, A & FR, 2663227, A	18, 22, 23, 27

Form PCT/ISA/210 (continuation of second sheet) (July 1992)